

## University of Groningen

### New insights into the biological role of COMMD1

Bartuzi, Paulina

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2014

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Bartuzi, P. (2014). *New insights into the biological role of COMMD1: from inflammation to steatosis and hypercholesterolemia*. [Thesis fully internal (DIV), University of Groningen]. [S.n.].

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

# CHAPTER 4

## Copper metabolism domain-containing 1 represses genes that promote inflammation and protects mice from colitis and colitis-associated cancer

*Li H<sup>1</sup>, Chan L<sup>1</sup>, Bartuzi P<sup>2</sup>, Melton SD<sup>3</sup>, Weber A<sup>4</sup>, Ben-Shlomo S<sup>5</sup>, Varol C<sup>5</sup>, Raetz M<sup>6</sup>, Mao X<sup>1</sup>, Starokadomskyy P<sup>1</sup>, van Sommeren S<sup>7</sup>, Mokadem M<sup>1</sup>, Schneider H<sup>8</sup>, Weisberg R<sup>1</sup>, Westra HJ<sup>9</sup>, Esko T<sup>10</sup>, Metspalu A<sup>10</sup>, Kumar V<sup>9</sup>, Faubion WA<sup>11</sup>, Yarovinsky F<sup>6</sup>, Hofker M<sup>2</sup>, Wijmenga C<sup>9</sup>, Kracht M<sup>4</sup>, Franke L<sup>9</sup>, Aguirre V<sup>1</sup>, Weersma RK<sup>7</sup>, Gluck N<sup>5</sup>, van de Sluis B<sup>2</sup>, and Burstein E<sup>1,12</sup>*

<sup>1</sup>Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas, <sup>2</sup>Department of Pediatrics (Section of Molecular Genetics),

University of Groningen, University Medical Center Groningen, Groningen, The Netherlands,

<sup>3</sup>Department of Pathology, Dallas VA Medical Center, Dallas, Texas, <sup>4</sup>Rudolf Buchheim Institute of Pharmacology, Justus Liebig University, Giessen, Germany, <sup>5</sup>Gastroenterology

Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, <sup>6</sup>Department of Immunology, University of Texas Southwestern Medical Center, Dallas, Texas,

<sup>7</sup>Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, <sup>8</sup>Institute of Physiological

Chemistry, Hannover Medical School, Hannover, Germany, <sup>9</sup>Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands,

<sup>10</sup>Estonian Genome Center, University of Tartu, Tartu, Estonia, <sup>11</sup>Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota, <sup>12</sup>Department of Molecular Biology,

University of Texas Southwestern Medical Center, Dallas, Texas

## ABSTRACT

**BACKGROUND & AIMS:** Activation of the transcription factor nuclear factor- $\kappa$ B (NF- $\kappa$ B) has been associated with the development of inflammatory bowel disease (IBD). Copper metabolism MURR1 domain containing 1 (COMMD1), a regulator of various transport pathways, has been shown to limit NF- $\kappa$ B activation. We investigated the roles of COMMD1 in the pathogenesis of colitis in mice and IBD in human beings.

**METHODS:** We created mice with a specific disruption of *Commd1* in myeloid cells (Mye-knockout [K/O] mice); we analyzed immune cell populations and functions and expression of genes regulated by NF- $\kappa$ B. Sepsis was induced in Mye-K/O and wild-type mice by cecal ligation and puncture or intraperitoneal injection of lipopolysaccharide (LPS), colitis was induced by administration of dextran sodium sulfate, and colitis-associated cancer was induced by administration of dextran sodium sulfate and azoxymethane. We measured levels of *COMMD1* messenger RNA in colon biopsy specimens from 29 patients with IBD and 16 patients without (controls), and validated findings in an independent cohort (17 patients with IBD and 22 controls). We searched for polymorphisms in or near *COMMD1* that were associated with IBD using data from the International IBD Genetics Consortium and performed quantitative trait locus analysis.

**RESULTS:** In comparing gene expression patterns between myeloid cells from Mye-K/O and wild-type mice, we found that COMMD1 represses expression of genes induced by LPS. Mye-K/O mice had more intense inflammatory responses to LPS and developed more severe sepsis and colitis, with greater mortality. More Mye-K/O mice with colitis developed colon dysplasia and tumors than wild-type mice. We observed a reduced expression of COMMD1 in colon biopsy specimens and circulating leukocytes from patients with IBD. We associated single-nucleotide variants near *COMMD1* with reduced expression of the gene and linked them with increased risk for ulcerative colitis.

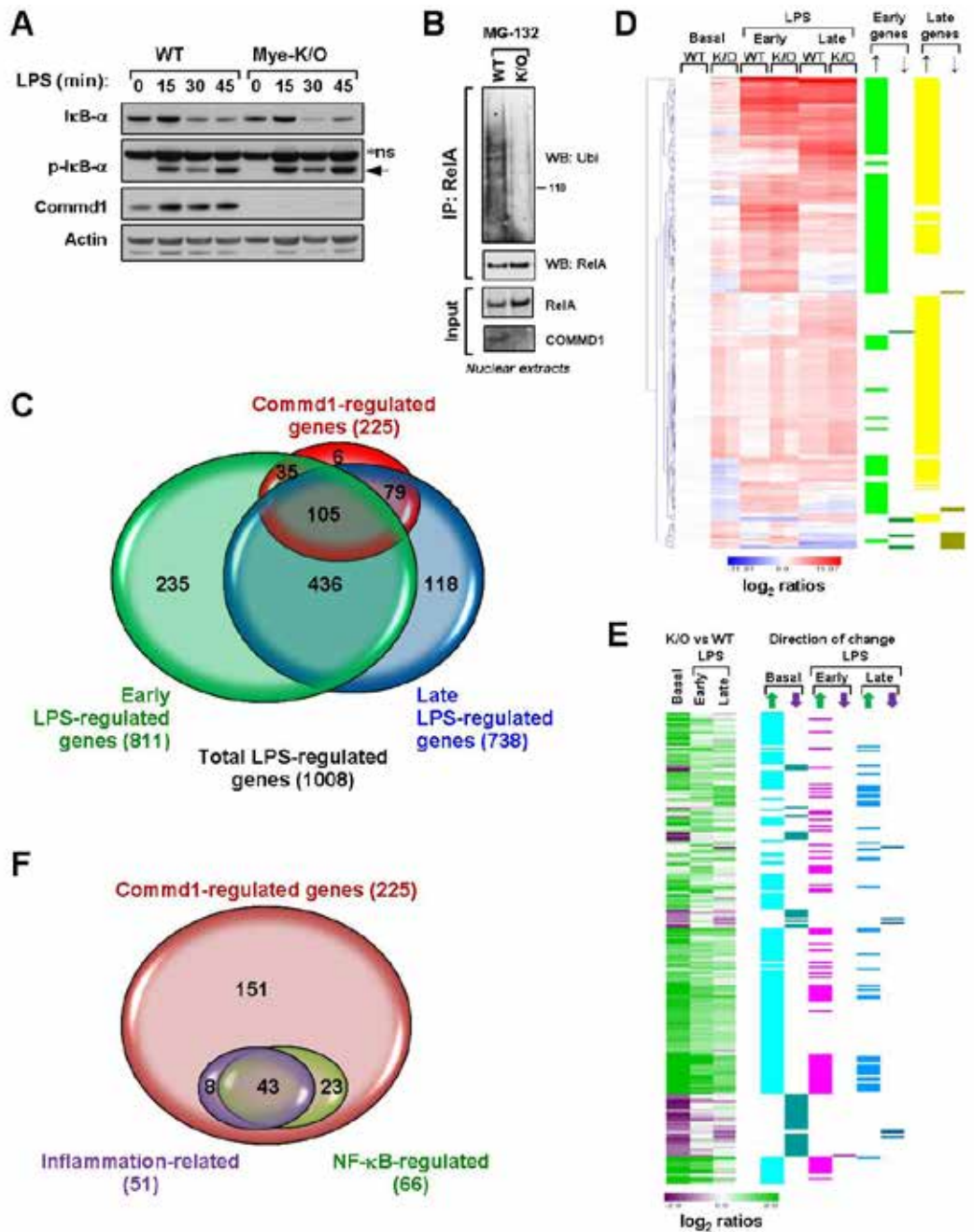
**CONCLUSIONS:** Expression of COMMD1 by myeloid cells has anti-inflammatory effects. Reduced expression or function of COMMD1 could be involved in the pathogenesis of IBD.

## INTRODUCTION

Persistent inflammation is a common maladaptive component in the pathogenesis of human diseases. A prime example of this paradigm is inflammatory bowel disease (IBD), a chronic inflammatory process of the intestinal tract that clinically presents as two phenotypic entities: ulcerative colitis (UC) and Crohn's disease (CD). This disorder involves an interaction between environmental factors and inherited susceptibility, and is associated with an increased risk for colorectal cancer [1].

The regulation of the inflammatory cascade is a complex process in which the transcription factor NF- $\kappa$ B plays a master regulatory role [2]. Consequently, this factor has also been linked to the pathogenesis of several chronic inflammatory conditions in human beings [2], including IBD [3, 4]. Canonical NF- $\kappa$ B activity is mediated primarily by NF- $\kappa$ B complexes containing the RelA subunit (also known as p65) or its paralog c-Rel. Under basal conditions, these complexes are kept in the cytosol through interactions with the inhibitory I $\kappa$ B proteins. Their activation requires I $\kappa$ B degradation, an event triggered by a critical kinase complex known as I $\kappa$ B kinase that sits at the cross-roads of numerous signaling pathways. After signaling inputs abate, homeostatic mechanisms that restore basal NF- $\kappa$ B activity are essential for the physiologic function of this pathway. The induction of I $\kappa$ B gene expression [5], or the expression of I $\kappa$ B kinase inhibitory proteins, such as A20 or CYLD [6, 7], participate in the timely termination of NF- $\kappa$ B activity. The expression of these factors is under the control of NF- $\kappa$ B itself, thus providing negative feedback loops in the pathway. In addition, it has been recognized that ubiquitination and proteasomal degradation of RelA is critical to terminate transcription of a variety of genes [8-13]. One ligase responsible for these effects contains the scaffold protein Cul2 in association with copper metabolism MURR1 domain containing 1 (COMMD1) [10, 12], a prototypical member of the COMMD protein family [14].

In addition to its role in NF- $\kappa$ B regulation [12, 15], COMMD1 has been implicated in a variety of cellular processes, including copper transport [16], electrolyte balance [17-19], and hypoxia responses [20]. Given these pleiotropic functions, it has remained unclear whether this factor plays a physiologically important role in the control of inflammation *in vivo* and whether it could play a role in chronic inflammatory diseases. Here we report that myeloid-specific deficiency of *Commd1* leads to more intense activation of LPS-inducible genes and is associated with more severe inflammation. In addition, we present genetic evidence linking gene variants associated with reduced *COMMD1* expression to risk for UC in humans, highlighting the physiologic importance of this gene in immunity and IBD pathogenesis.



**Figure 1: *Commd1* regulates the LPS transcriptional program in myeloid cells.** (A) *Commd1* does not affect  $\text{IkB}$  turnover in BMDMs.  $\text{IkB-}\alpha$  phosphorylation (p- $\text{IkB-}\alpha$ ) and degradation after LPS treatment were examined by Western blotting. \*ns, nonspecific band (arrow points to p $\text{IkB}\alpha$ ) (B) *Commd1* deficiency reduces RelA ubiquitination. *Commd1*-deficient mouse embryo fibroblasts (K/O) or control cells (WT) were used to detect ubiquitinated RelA by ubiquitin immunoblotting of immunoprecipitated RelA. (C) Gene expression differences resulting from *Commd1* deficiency substantially overlap with the LPS response. BMDMs from WT and Mye-K/O mice were utilized in duplicate microarray analysis to ascertain genes regulated by *Commd1* or by LPS (100ng/mL)

## RESULTS

### COMMD1 REPRESSES PROINFLAMMATORY GENE EXPRESSION IN MYELOID CELLS

To evaluate the potential role of COMMD1 in inflammation, and given the known embryonic lethality that results from complete *Commd1* deficiency in mice [23], we generated a tissue-specific mouse model of *Commd1* deficiency [24]. First, *Commd1* was selectively deleted in myeloid cells (Mye-knockout [K/O]), a critical lineage in innate immunity, leading to the expected loss of *Commd1* expression in macrophages (Supplementary Figure 1A). Mye-K/O mice were healthy and B lymphocyte (B220<sup>+</sup>) and T lymphocyte populations (CD3<sup>+</sup> and CD4/CD8) were not significantly different in the spleen or mesenteric lymph nodes (Supplementary Figure 1B and C). Similarly, myeloid populations, including granulocytes (Ly6G<sup>+</sup>), monocytes and macrophages (CD11b<sup>+</sup>, Ly6C<sup>+</sup> and F4/80), and dendritic cells (CD11c<sup>high</sup>, and CD11c<sup>intermediate</sup>) were not significantly different in Mye-K/O mice (Supplementary Figure 1B-D). In line with previous observations [12, 25, 26], *Commd1* deficiency did not alter substantially the phosphorylation or turnover of IκB (Figure 1A), but had a profound effect on RelA ubiquitination (Figure 1B).

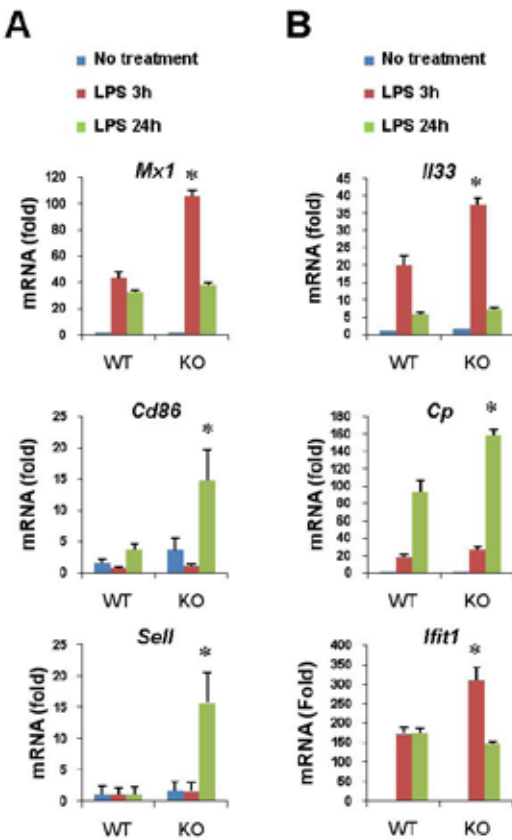
Next, using bone marrow-derived myeloid cells (BMDMs) from the Mye-K/O mice, we assessed the impact of *Commd1* on the LPS transcriptional response at a genome-wide level. High-density microarray experiments indicated that 1008 genes were regulated by LPS at least 3fold in two independent series of experiments (Figure 1C, and Supplementary Tables 1-3). In addition, the expression of 225 genes was found to be regulated by *Commd1* (Supplementary Tables 1 and 2). Notably, the vast majority of *Commd1*-regulated genes (219 of 225) was also regulated by LPS, and only few *Commd1* target genes (6 out of 225) were outside the LPS transcriptional response (Supplementary Tables 2 and 3). Hierarchical clustering of these 225 genes was used to visualize the pattern of deregulated expression in *Commd1*-deficient myeloid cells. Both early and late LPS-inducible genes were affected by *Commd1* deficiency (Figure 1D). In most instances, the changes observed consisted of increased gene expression, which often were noted even at basal levels (Figure 1E).

To further understand the effect of *Commd1* on gene expression in myeloid cells, we performed a functional analysis using the Kyoto encyclopedia of genes and genomes (KEGG) database. This showed that only 51 genes (23%) participate in immune regulation

---

at early (3h) or late (24h) time points. The degree of overlapping genes is presented in Venn diagram form. (D) Hierarchical cluster analysis of *Commd1*-regulated genes during the LPS response. Expression levels normalized to basal expression in WT samples is shown as color-coded log<sub>2</sub>-transformed ratios. The gene induction pattern, either early (>3-fold induction at 3h) or late LPS response (>3-fold induction at 24h), is also indicated. The specific effect of *Commd1* deficiency is noted by *up arrows* or *down arrows*. (E) The genes analyzed in panel D are reanalyzed by calculating ratios between K/O and WT BMDMs, displayed as color-coded log<sub>2</sub>-transformed manner. The direction of change is indicated in adjacent columns by *up arrows* or *down arrows*. (F) Venn diagram of *Commd1*-regulated genes, indicating those involved in inflammatory responses or regulated by NF-κB.

regulation (as defined by any of a number of KEGG entries) (Supplementary Table 4), indicating that a large number of *Commd1* gene targets in myeloid cells are not known inflammatory regulators (Figure 1F). Next, we assessed the contribution of NF- $\kappa$ B regulation to the effects noted in *Commd1*-deficient myeloid cells. To that end, NF- $\kappa$ B regulation of genes of interest was ascertained through 2 overlapping approaches: first, using a curated database of published NF- $\kappa$ B gene targets (publicly available at [www.NF-kB.org](http://www.NF-kB.org)), and second, using chromatin immunoprecipitation (ChIP) for RelA followed by sequencing (ChIP-seq) in LPS-stimulated BMDMs [27]. This indicated that only 66 genes regulated by *Commd1* (29%) (Supplementary Table 4) are known NF- $\kappa$ B or RelA targets, suggesting that a substantial proportion of the effects of *Commd1* are either secondary or mediated through other transcriptional regulators.



**Figure 2: Gene selective effects of *Commd1* on NF- $\kappa$ B regulated targets.** BMDMs (2 mice per group, with triplicate cultures per mouse) were stimulated with LPS and gene expression was determined by quantitative reverse-transcription polymerase chain reaction. (A) Selected NF- $\kappa$ B target genes are shown, and other (B) LPS-inducible genes that are not NF- $\kappa$ B targets are also shown.

### COMMD1 EXERTS GENE-SELECTIVE EFFECTS AMONG NF-KB-REGULATED TARGETS

Altogether, these findings suggest a substantial repressive role for *Commd1* on the LPS response in myeloid cells and confirmed a negative regulatory role for *Commd1* on selected NF- $\kappa$ B-regulated genes. Indeed, quantitative reverse-transcription polymerase chain reaction analysis corroborated that certain NF- $\kappa$ B target genes were selectively regulated (for example, *Mx1*, *Cd86* and *Sell*) (Figure 2A), whereas others were relatively insensitive to *Commd1* deficiency (such as *Il6*, *Tnfaip3* and *Mmp13*) (Supplementary Figure 2A).

These findings are in line with previous reports of gene-specific effects for COMMD1 in cancer cell lines [12]. Enhanced expression of selected genes that are not directly regulated by NF- $\kappa$ B was confirmed as well (Figure 2B). Interestingly, in contrast to these effects, several hypoxia-induced genes were not affected by *Commd1* deficiency in BMDMs (Supplementary Figure 2B), despite its reported role in hypoxia-dependent gene expression [20, 23]. In aggregate, the data indicate that *Commd1* primarily exerts a gene-selective repressive function on the LPS response.



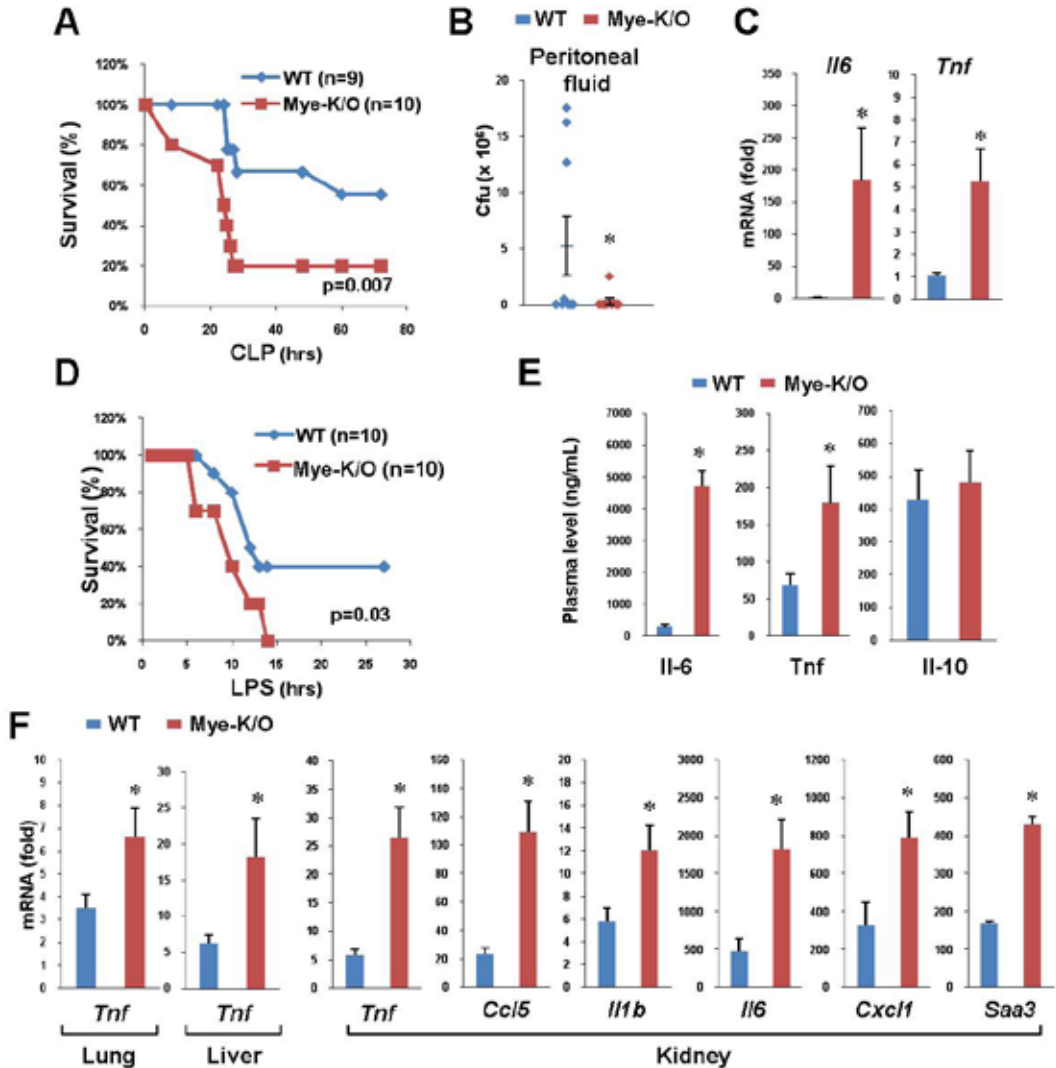
## MYELOID DEFICIENCY OF COMMD1 LEADS TO EXAGGERATED INFLAMMATORY RESPONSES IN VIVO

In view of the effects of *Commd1* on LPS-induced gene expression, we used 2 models to contrast the inflammatory response of Mye-K/O mice and wild-type (WT) littermate controls. First, we used the cecal ligation and puncture (CLP) model of polymicrobial sepsis. Animal survival was compromised in Mye-K/O mice compared with WT controls (Figure 3A). In contrast, cultures of peritoneal fluid showed lower bacterial counts in the Mye-K/O mice (Figure 3B), indicating that their increased mortality was not caused by greater bacterial load. Rather, after accounting for bacterial invasion (by examining animals with negative bacterial cultures), we found that Mye-K/O mice had increased cytokine expression (Figure 3C), suggesting that exaggerated proinflammatory gene expression was responsible for their increased susceptibility to sepsis. Next, animals were challenged directly with intraperitoneal LPS injection. Once more, Mye-K/O mice showed greater mortality (Figure 3D) and increased morbidity (Supplementary Figure 3A). Tissue injury, manifested by lactate dehydrogenase (LDH) release in plasma, also was more pronounced in Mye-K/O mice (Supplementary Figure 3B) which also displayed greater plasma increases of interleukin (Il)6 and tumor necrosis factor (Tnf) (Figure 3E). In contrast, plasma levels of the anti-inflammatory cytokine Il10 were comparable with WT. Tissue expression of proinflammatory genes was more pronounced in Mye-K/O mice, as exemplified by *Tnf*, as well as other proinflammatory genes (Figure 3F and Supplementary Figure 4). Altogether, these data indicate that *Commd1* deficiency in the myeloid lineage leads to more profound acute inflammatory responses.

## REDUCED EXPRESSION OF COMMD1 IS A COMMON FEATURE OF IBD

Given the findings made in the Mye-K/O mouse model, we decided to explore whether *COMMD1* could play a role in the pathophysiology of inflammatory disorders in human beings, choosing IBD as a disease model. To this end, we evaluated the expression of *COMMD1* in IBD patients. We found that *COMMD1* messenger RNA (mRNA) levels in colonic endoscopic biopsy specimens were reduced in an Israeli cohort of patients with colitis (n=29, 18 with Crohn's disease and 11 with ulcerative colitis) compared to unaffected controls (n=16; P=0.04) (Figure 4A, left). This finding was replicated in an independent patient cohort in the United States (P= 0.04, 22 normal controls and 17 patients with IBD, 9 of whom had CD and 8 had UC) (Supplementary Figure 5). Similarly, we found that *COMMD1* mRNA was reduced in circulating leukocytes from IBD patients with active disease (n=12, 9 with CD and 3 with UC) compared with normal individuals (n=14; P=0.001) (Figure 4A, right). Moreover, in a murine model of colitis induced by dextran sodium sulfate (DSS) administration, we also observed decreased mRNA and protein expression of *Commd1* and other *Commd* genes in the colon (Figure 4B and C). In aggregate, the data indicate that mucosal inflammation is associated with decreased *COMMD1* gene expression in clinical and experimental situations.





**Figure 3: Mye-K/O mice have a more severe sepsis response.** (A) Cecal ligation and puncture leads to a higher mortality rate in Mye-K/O mice. Mye-K/O animals (*Comm1<sup>E/F</sup>*, *LysM-Cre*) were compared with wild-type (WT) littermate controls (*Comm1<sup>E/F</sup>*). Survival without need for euthanasia was plotted and the Kaplan-Meier estimator was calculated. (B) Peritoneal cultures yield lower bacterial counts in Mye-K/O mice (9 mice in each group). Colony forming units (Cfu) in blood agar were determined by progressive dilution. (C) Induction of proinflammatory gene expression is greater in Mye-K/O mice. Tissue RNA was analyzed by quantitative reverse-transcription polymerase chain reaction (in kidney). To normalize for bacterial load, animals with no detectable bacterial counts were used for this analysis (WT, 4 mice; Mye-K/O, 6 mice). (D) Mye-K/O mice have greater mortality after LPS administration. Survival was analyzed as in panel A. (E) Plasma levels of *Il6*, *Tnf* and *Il10* were determined in Mye-K/O mice (n=9) and WT controls (n=10) at the time of euthanasia in the LPS model. (F) LPS induction of proinflammatory genes was determined by quantitative reverse-transcription polymerase chain reaction in tissues of Mye-K/O mice (n=10) and WT controls (n=10). \*  $P < 0.05$ .

## GENETIC VARIANTS ASSOCIATED WITH DECREASED COMMD1 EXPRESSION ARE ASSOCIATED WITH UC RISK

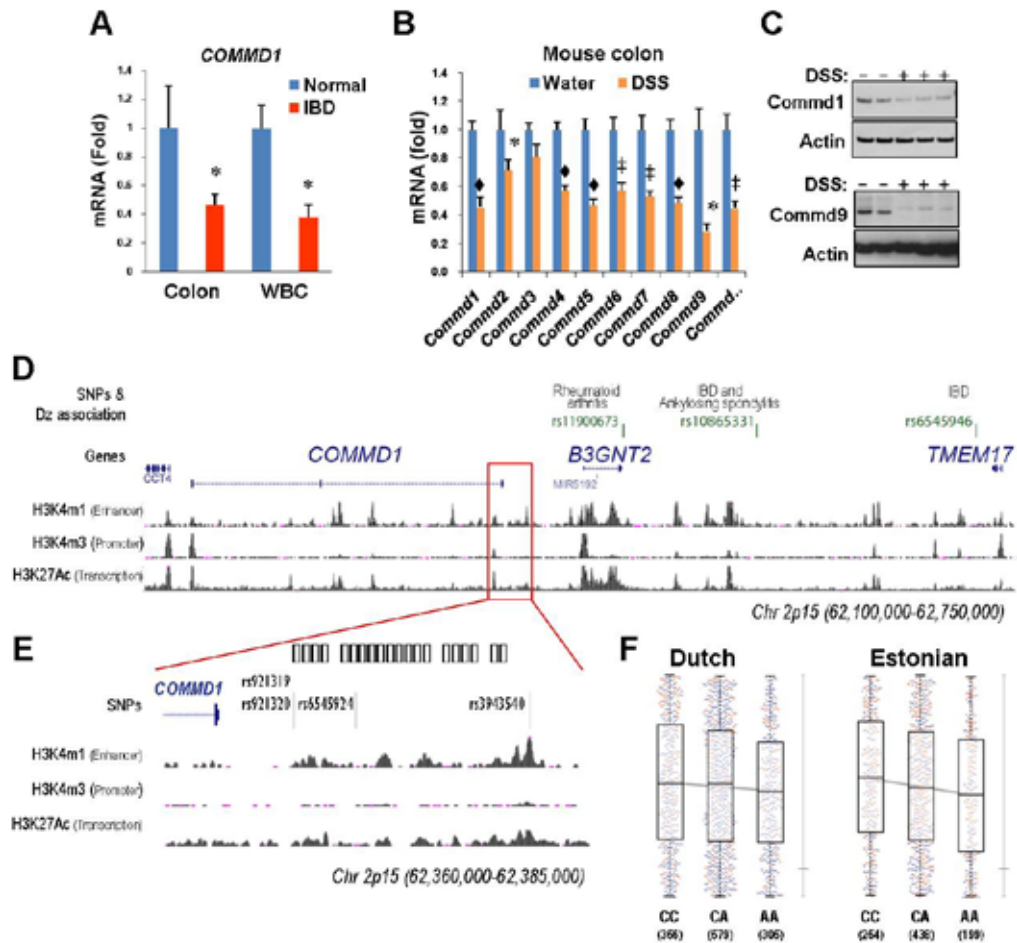
In view of the reduced expression of *COMMD1* in IBD patients, we investigated whether genetic polymorphisms of this gene might affect IBD risk in human beings. Interestingly, genome-wide association studies (GWAS) have linked an intergenic region near *COMMD1* to the risk for various inflammatory conditions including IBD (Figure 4D) [3, 28-32], but the precise gene responsible for these effects remains unknown.

We began by interrogating the most recent meta-analysis of genome-wide association studies (GWAS) for CD and UC [3] by the IIBDGC, which is currently available on the Ricopili server ([www.broadinstitute.org/mpg/ricopili/](http://www.broadinstitute.org/mpg/ricopili/)). These data indicated a suggestive association signal with UC for single-nucleotide polymorphisms (SNPs) located just downstream of *COMMD1*, including rs3943540 ( $P=7.89 \times 10^{-5}$ ) and rs921319 ( $P=7.47 \times 10^{-5}$ ) (Figure 4E). Interestingly, both SNPs are in linkage disequilibrium (LD) with each other ( $r^2=1$ ,  $D'=1$ ). Additional analysis of the 1000 genomes-imputed IIBDGC UC and CD meta-analyses also showed a suggestive association with UC for a SNP located in the same region, rs6545924 ( $P=2.27 \times 10^{-5}$ , odds ratio (OR)=1.104, 95% confidence interval of the OR between 1.06-1.15).

Epigenetic analysis in myeloid cells performed by the Encyclopedia of DNA Elements (ENCODE) project [33], showed that this genomic locus displays features of a gene regulatory region (Figure 4D and E), such as histone 3 lysine 4 monomethylation (H3K4me1) and lysine 27 acetylation (H3K27Ac), both associated with active enhancers [34]. In agreement with this notion, the SNP and CNV Annotation (SCAN) database [35] indicates that there is a significant eQTL effect of rs921319 on *COMMD1* gene expression. To further examine the possibility that these variants have an effect on *COMMD1* expression, we conducted *cis*-eQTL mapping on whole peripheral blood of 2 cohorts (1240 samples previously studied [21] and 891 samples from the Estonian Biobank). Because the SNPs in question were not present in our eQTL data set, we tested rs921320, located in very close proximity to rs921319 and a perfect proxy SNP for rs6545924 ( $r^2=0.9$ ,  $D'=1$ ). We observed a significant eQTL effect of rs921320 on *COMMD1* expression, with the same allelic direction for the Dutch and Estonian cohorts (the *P* values for individual cohorts were 0.057 and 0.0099, respectively; with a weighted Z-method meta-analysis  $P=0.0033$ ). The A-allele of rs921320, which is linked to the risk allele for rs6545924, was associated with reduced expression of *COMMD1* (Figure 4F). On the other hand, we did not observe any eQTL effects of rs921320 on the 2 other neighboring genes, *B3GNT2* or *TMEM17*. Altogether, these findings suggest that genetic variation in the 3' region of *COMMD1*, which has regulatory effects on gene expression, is linked to risk for UC.

## MYELOID CELL DEFICIENCY OF COMMD1 LEADS TO MORE SEVERE COLITIS

To further examine the possible involvement of *COMMD1* in IBD pathogenesis, we began by assessing the *in situ* expression of this gene in the colon. Immunofluorescence staining of mouse colon tissue showed staining in the epithelium as well as in mononuclear cells in the lamina propria (Supplementary Figure 6). Furthermore, confocal microscopy images confirmed that



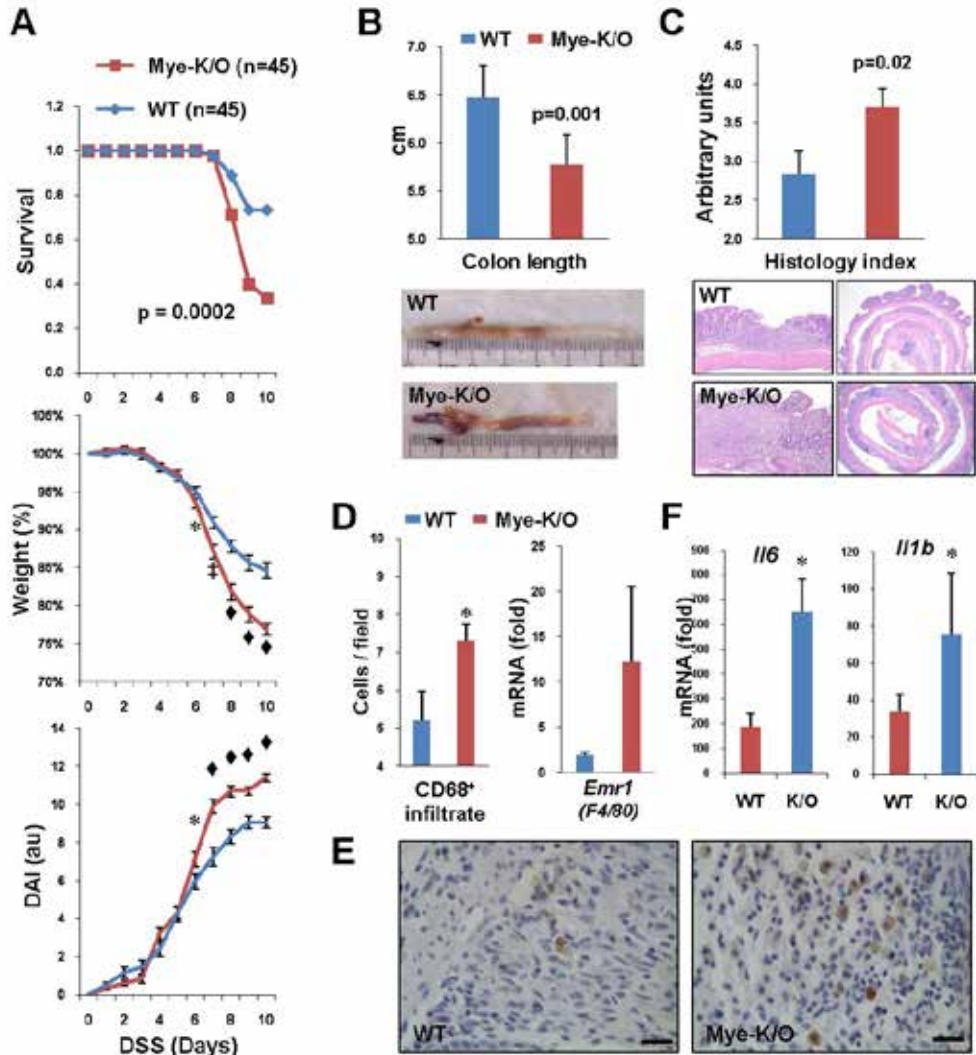
**Figure 4: Lower *COMMD1* expression is linked to IBD.** (A) *COMMD1* gene expression is suppressed in IBD patients. *COMMD1* mRNA expression was determined by quantitative reverse-transcription polymerase chain reaction in endoscopic colonic biopsies from IBD patients with colitis (n=29, 18 with CD and 11 with UC) and normal controls (n=17). Similarly, *COMMD1* mRNA levels in white blood cells (WBCs) were determined in IBD patients (n=12, 9 with CD and 3 with UC) and unaffected controls (n=14). (B and C) *Commmd* gene expression at the (B) mRNA level was decreased similarly in colonic tissue after DSS-induced acute colitis (7 mice in the DSS group compared with 8 mice in the water group), and (C) this was shown at the protein level for *Commmd1* and *Commmd9* (C). \* $P < 0.05$ , \* $P < 0.001$ , \* $P < 0.0001$ . (D) Polymorphisms near *COMMD1* are associated with UC risk. A map of the 2p15 locus, including SNPs previously implicated in inflammatory diseases, is shown. The region located 3' of the *COMMD1* gene, where SNPs associated to UC were found, is marked by a red box. (E) A closer view of the location of the SNPs of interest downstream of *COMMD1* is shown, including chromatin modifications such as histone 3 K4 monomethylation (H3K4m1), trimethylation (H3K4m3) and histone 3 K27 acetylation (H3K27Ac). (F) *cis*-eQTL analysis for rs921320 in 2 independent cohorts. *COMMD1* gene expression (in the y axis) is shown in a box plot for all 3 genotypes for this SNP (CC, CA or AA). The horizontal bar in the box is the median, the top and bottom of the box show the interquartile range (IQR) (Q3 - Q1); the whiskers show the quartile + 1.5x the IQR. The number of individuals in each group is indicated (under the genotype); men and women are denoted by blue and red dots, respectively.

resident lamina propria macrophages (F4/80<sup>+</sup>) are also COMMD1 positive, in agreement with Western blot analysis of primary myeloid cells (Figure 1A). With this in mind and in view of the known contributions of both epithelial and myeloid cells in the pathogenesis of human and murine colitis [36, 37], we evaluated the role of this gene in this disease process using both epithelial and myeloid cell-specific knockout mice.

First, we used the DSS model, which triggers colonocyte injury and an innate immune response to luminal bacteria that results in acute colitis. Mye-K/O mice showed more severe disease than WT animals, with excess mortality, more profound weight loss, and worse disease activity (Figure 5A). In agreement with this, colon shortening (a macroscopic marker of tissue injury) and histologic evaluation of the colonic mucosa both indicated more damage in Mye-K/O mice (Figures 5B and C). In contrast to these results, *Comm1d1* inactivation in the intestinal epithelium did not result in any appreciable alteration of DSS-induced colitis (Supplementary Figure 7). The more severe inflammatory response in Mye-K/O mice was correlated with greater numbers of CD68<sup>+</sup> myeloid cells in the colon as seen by immunohistochemistry (Figures 5D, left panel, and E), and a consistent trend was observed at the mRNA level for the macrophage-specific gene *Emr1* (F4/80, Figure 5D, right panel). In addition, Mye-K/O mice showed increased expression of proinflammatory genes, such as *Il6* and *Il1b* (Figure 5F). However, myeloid and lymphoid populations in the mesenteric lymph node (MLN) (Supplementary Figure 8) and the spleen (not shown), were not significantly different between the groups.

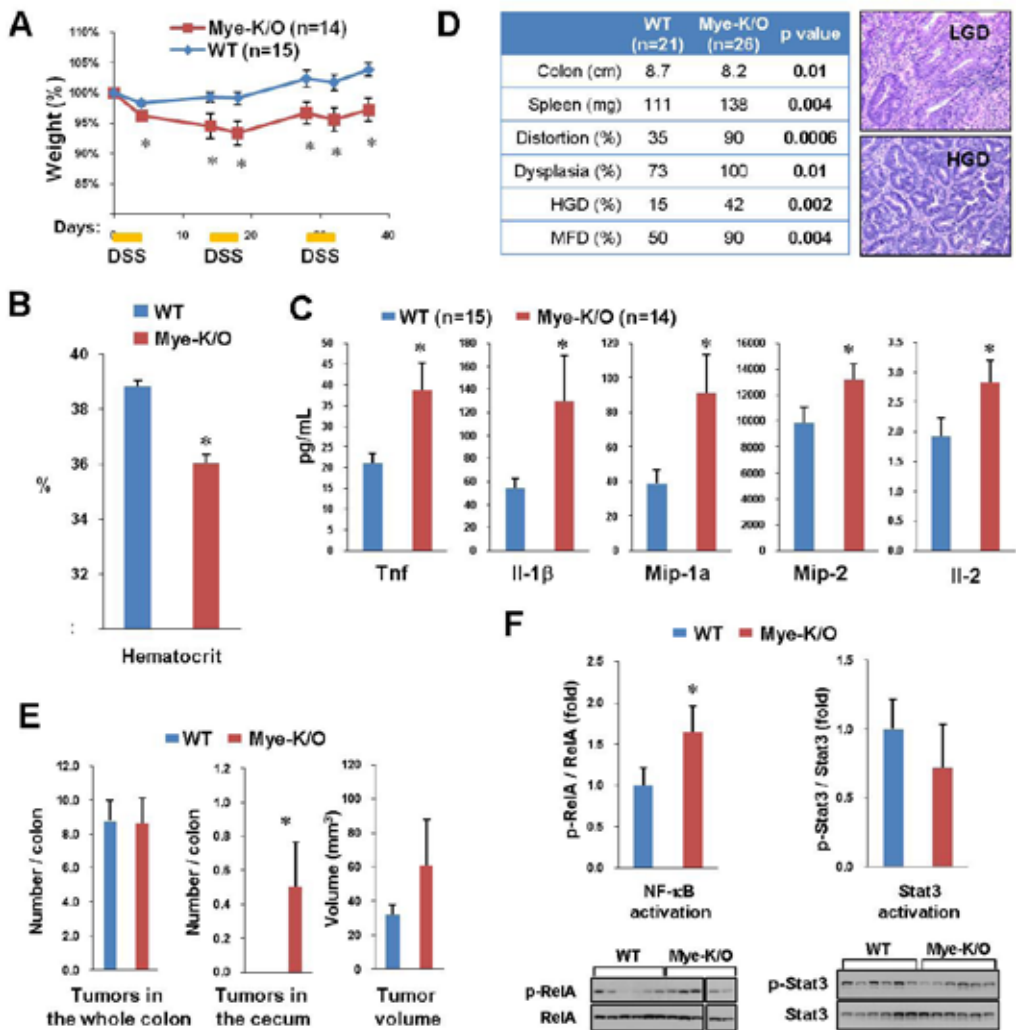
#### MYELOID CELL DEFICIENCY OF COMMD1 PROMOTES COLITIS-DYSPLASIA PROGRESSION

Further analysis indicated that recurrent DSS administration, which mimics the chronic nature of IBD, also resulted in more severe disease in Mye-K/O mice, manifested as greater weight loss (Figure 6A) and more severe anemia (Figure 6B). Colonic tissue from these animals also produced greater amounts of several proinflammatory factors such as Tnf (Figure 6C and S9). In addition, azoxymethane (AOM) followed by recurrent DSS treatments was used to examine progression to dysplasia. In this model, Mye-K/O mice also showed more severe disease, as evidenced by more severe colonic shortening, splenomegaly, and histologic distortion of the mucosal architecture (Figure 6D). Moreover, after 7 weeks of treatment, Mye-K/O mice had a greater propensity to develop dysplasia, high-grade dysplasia (HGD) or multifocal dysplasia (MFD) as assessed histologically (Figure 6D). By 10 weeks, small adenomas could be visualized, but the total number of lesions was not increased in Mye-K/O mice (Figure 6E). Nevertheless, only Mye-K/O mice developed proximal colonic tumors and there was a tendency for the tumor burden to be greater in these animals as a result of greater adenoma volume (Figure 6E). In colonic tissue, Mye-K/O animals showed greater NF-κB activation as evidenced by the ratio of the transcriptionally active form of RelA (phosphorylated at Ser536) to total RelA. However, STAT3 activation, another pathway known to promote cancer in the context of chronic tissue inflammation [38], was not altered in these mice (Figure 6F). Altogether, these data highlight a role for myeloid cell expression of *Comm1d1* in chronic colonic inflammation and progression to dysplasia.



**Figure 5: Myeloid deficiency of *Comm1* predisposes to more severe colitis.** (A) Worse survival, weight loss, and disease activity index (DAI) were noted in Mye-K/O mice compared with wild-type littermate controls during acute DSS-induced colitis. \* $P < 0.05$ , \* $P < 0.001$ , \* $P < 0.0001$ . (B) Colon length was significantly shorter in Mye-K/O mice ( $n=26$ ) after acute colitis compared to WT controls ( $n=20$ ). Representative images are shown. (C) Worse tissue injury was also evident histologically. By using a severity score, colonic sections were examined (11 Mye-K/O and 9 WT mice). Representative histologic sections are shown. (D and E) Expansion of macrophage populations in the colonic mucosa of Mye-K/O mice. In the *left panel*, the number of CD68<sup>+</sup> cells present in the mucosa was assessed by immunohistochemistry in a tissue microarray of DSS colitis samples (Mye-K/O, 13 samples; WT, 12 samples). In the *right panel*, the colonic expression of the macrophage marker gene *Emr1* (*F4/80*) was determined by quantitative reverse-transcription polymerase chain reaction (Mye-K/O, 18 samples; WT, 23 samples). (E) Representative images of the CD68 immunohistochemistry are shown. Scale bar: 20  $\mu$ m. (F) RNA extracted from colonic tissue was used for quantitative reverse-transcription polymerase chain reaction analysis for the genes indicated (Mye-K/O, 18 samples; WT, 23 samples). au: arbitrary units.





**Figure 6: *Commd1* deficiency leads to worse chronic colitis and transition to dysplasia.** (A) Recurrent administration of DSS caused more weight loss in Mye-K/O mice. (B) Chronic colitis led to more severe anemia in Mye-K/O mice (5 mice per group). (C) Pro-inflammatory cytokine production is increased in colonic tissue of Mye-K/O mice. Colonic full-thickness tissue harvested from colitic mice was cultured overnight and the secretion of cytokines into the growth media was determined. (D) Progression to dysplasia was worse in Mye-K/O animals. Azoxymethane (AOM) was administered before chronic colitis induction and dysplasia was assessed histologically at 7 weeks. Representative images are displayed. (E) Tumor number and volume in mice at 10 weeks of colitis/dysplasia induction is shown. \* $P < 0.05$ . (F) Greater NF- $\kappa$ B activation in inflamed colonic tissue of Mye-K/O mice. Activation of NF- $\kappa$ B/RelA and Stat3 was assessed in inflamed colonic tissue of animals treated with AOM / DSS. Western blot analysis was performed with tissue lysates and the active phosphorylated form was quantitated and normalized to expression of the respective target. HGD, high-grade dysplasia; LGD, low-grade dysplasia; MFD, multifocal dysplasia.

## DISCUSSION

COMMD1 plays an essential role to restrain the activity of NF- $\kappa$ B, a master regulator of inflammation. Specifically, COMMD1 acts to terminate NF- $\kappa$ B transcriptional activation through the ubiquitin-mediated degradation of NF- $\kappa$ B subunits. A detailed molecular mechanism by which COMMD1 restrains NF- $\kappa$ B has been examined and reported previously [8, 10, 12, 13]. The studies presented here confirm the negative regulatory role of this factor on the NF- $\kappa$ B pathway [10, 12, 13, 15], and also underscores the gene-selective nature of these effects. Moreover, the studies in this model corroborate that key aspects of the reported mechanism are evident in the knockout mice, namely normal I $\kappa$ B phosphorylation and turnover, but diminished ability to terminate NF- $\kappa$ B transcriptional activity (ie, reduced RelA/p65 ubiquitination). Importantly, this study investigates the physiological relevance of these events in disease pathogenesis. The data indicate that COMMD1 expression in the myeloid compartment plays an important role in the regulation of inflammatory responses *in vivo*. Given that diverse functions have been reported for this gene [17, 39-41], this report fills a critical void by underscoring that COMMD1 participates in immune regulation. In addition, the results also suggest that COMMD1 has broader effects on proinflammatory gene expression programs that might extend beyond the NF- $\kappa$ B pathway. Whether these effects are the result of secondary perturbations or are the result of direct regulation of transcription factor(s) other than NF- $\kappa$ B, should be further examined in the future.

In the context of intestinal inflammation and progression to cancer, previous studies have underscored the importance of epithelial and myeloid cells in these processes [14, 37, 38]. In particular, epithelial intrinsic NF- $\kappa$ B activation has been shown to be a protective response that promotes cell survival and barrier function [42], and myeloid cell NF- $\kappa$ B activation is important in sustaining the inflammatory response [36]. The increased propensity to inflammation and colitis observed as a result of myeloid cell deficiency of *Commd1* is in agreement with this paradigm and suggests that the immune regulatory roles of this gene are physiological. In addition, the increased progression to dysplasia in *Mye-K/O* mice is in agreement with the role of myeloid cells in promoting tumor growth through various proinflammatory cytokines [43]. In this regard, our model displays increased production of Tnf and greater tissue activation of NF- $\kappa$ B, both of which have been shown to promote cancer progression in the setting of colitis [36, 44]. In addition, we also found no appreciable phenotype of enterocyte-specific deficiency of *Commd1*, which stands in contrast to other models of disrupted NF- $\kappa$ B activity in the epithelium [36, 42]. This may reflect the fact that unlike other models, cell survival genes are not regulated by COMMD1 in the transcriptome analysis presented here. Furthermore, it suggests that other reported roles of *Commd1*, such as the regulation of various transport mechanisms, may be more relevant in these cells.

These observations in the animal model, coupled with the finding that genetic variants linked to lower *COMMD1* expression also are associated with an increased risk



for UC, suggest that this gene participates in the pathogenesis of this disorder in humans. In this context, it is important to note a recent report of a risk association for *COMMD7*, another member of the *COMMD* gene family [45]. Moreover, *COMMD1* acts in concert with *Cul2* to promote RelA ubiquitination [10, 12, 13], and the *CUL2* gene has been linked to IBD susceptibility [46]. Additional studies, including ongoing resequencing efforts, will be required to validate the genetic association seen here with greater statistical confidence. However, the aggregate of our data and published reports suggest the possibility that homeostatic mechanisms that restore NF- $\kappa$ B to its basal state, when decreased, may enhance the risk for IBD.

Our studies also identified that inflammation can lead to suppressed expression of *COMMD1*, as seen in patients with colitis as well as in animal models. Our data suggest that this is likely the consequence rather than the cause of inflammation in human disease, because this suppression can be induced by inflammation itself in animal studies. The decrease in *COMMD1* expression probably plays a physiologic role in the initiation of an appropriate inflammatory response, but in view of the more severe inflammatory responses seen when this gene is deleted in myeloid cells, persistent *COMMD1* suppression is probably maladaptive during chronic inflammation. Therefore, this work highlights the need to gain a refined understanding of the molecular events that control *COMMD1* gene expression in the setting of inflammation. With this knowledge, we may begin to envision potential interventions that might restore gene expression, which could be tested for their therapeutic potential in chronic inflammatory disorders.

## MATERIALS AND METHODS

**Human studies:** All procedures involving human subjects were reviewed and approved by the respective Institutional Review Boards (at UT Southwestern Medical Center, The Mayo Clinic and Tel-Aviv Sourasky Medical Center). Circulating leukocytes and intestinal biopsies were obtained at the time of endoscopy as part of the patients' ongoing medical care.

**Genome-Wide Association Studies and Quantitative Trait Locus Analysis:** Genetic association data were obtained from the 1000 genomes imputed meta-analysis from 15 genome-wide association studies of CD and/or UC conducted by the International IBD Genetic Consortium (IIBDGC) [3]. We conducted *cis*-expression quantitative trait locus (*cis*-eQTL) mapping on whole peripheral blood of 2 cohorts: 1240 samples from a previously published study [21] and 891 samples from the Estonian Biobank, both hybridized to Illumina (San Diego, CA) HT12-v3 oligonucleotide arrays using methodology as described in detail elsewhere [22].

**Statistical analysis:** In all graphs, the mean is presented and the error bars correspond to SEM. Statistical comparisons between mean values were performed using a 1-tailed,

heteroscedastic, Student *t* test. For nonparametric variables, the chi-square test was used. Survival curves were examined using the Kaplan-Meier analysis.

**Supplementary materials and methods:** All other materials and methods are described in the Supplementary Materials & Methods section.

Note: To access the supplementary material accompanying this article, visit the online version of Gastroenterology at [www.gastrojournal.org](http://www.gastrojournal.org), and at <http://dx.doi.org/10.1053/j.gastro.2014.04.007>.

## ACKNOWLEDGEMENTS

The authors are grateful to Eric Fearon for kindly providing some reagents used here. The authors are also grateful to John Shelton, Christine Komarck and Baozhi Chen for technical support for this project.

**Conflict of interest:** The authors disclose no conflicts.

**Funding:** The work of E.B. was supported by a National Institutes of Health grant (R01 DK073639), a CCFA Senior Research Award (SRA # 2737), a Cancer Prevention & Research Institute of Texas grant (CPRIT RP130409), and the Disease Oriented Clinical Scholars' Program at UT Southwestern. R.K.W was supported by supported by a VIDI grant (91713308) from the Netherlands Organization for Scientific Research (NWO). The work of N.G. was supported by a start-up grant from the U.S.-Israel Binational Science Foundation (BSF#2009339) and a grant from the German Israeli Foundation. The Estonian Genome Center at the University of Tartu (T.E. and A.P.) received financing from FP7 programs (ENGAGE, OPENGENE), targeted financing from the Estonian Government (SF0180142s08), Estonian Research Roadmap through the Estonian Ministry of Education and Research, Center of Excellence in Genomics (EXCEGEN) and University of Tartu (SP1GVARENG).

**Author contributions:** HL was responsible for the bulk of the experimental animal data performed here, including experiment execution and data analysis. LC, PB, RW, XM and PS participated in the colitis and colitis-associated cancer mouse models. SDM performed the pathological analysis of the tissue samples. AW, HS and MK performed the microarray experiments and analysis of myeloid cell LPS responses. SB-S, CV, WAF, VK and NG performed the gene expression analysis in human subjects. MM, MR, FY and VA participated in the CLP experiments. SVS and RKW performed the GWAS analysis. H-JW, TE, AM and LF performed the *cis*-eQTL analysis. MH, CW and BVDS were responsible for generating the mouse model and participated in specific portions of experimental design and interpretation. EB was responsible for the overall study design, data interpretation, and manuscript writing.

## REFERENCES

- [1] Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol* 2010;28:573-621.
- [2] Hayden MS, Ghosh S. NF- $\kappa$ B, the first quarter-century: remarkable progress and outstanding questions. *Genes Dev* 2012;26:203-34.
- [3] Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012;491:119-24.
- [4] Lees CW, Barrett JC, Parkes M, et al. New IBD genetics: common pathways with other diseases. *Gut* 2011;60:1739-1753.
- [5] Hoffmann A, Levchenko A, Scott ML, et al. The I $\kappa$ B-NF- $\kappa$ B signaling module: temporal control and selective gene activation. *Science* 2002;298:11241-1245.
- [6] Kovalenko A, Chable-Bessia C, Cantarella G, et al. The tumour suppressor CYLD negatively regulates NF- $\kappa$ B signalling by deubiquitination. *Nature* 2003;424:801-805.
- [7] Wertz IE, O'Rourke KM, Zhou H, et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF- $\kappa$ B signalling. *Nature* 2004;430:694-699.
- [8] Natoli G, Chiocca S. Nuclear ubiquitin ligases, NF- $\kappa$ B degradation, and the control of inflammation. *Sci Signal* 2008;1:pe1.
- [9] Sacconi S, Marazzi I, Beg AA, et al. Degradation of promoter-bound p65/RelA is essential for the prompt termination of the nuclear factor  $\kappa$ B response. *J Exp Med* 2004;200:107-113.
- [10] Mao X, Gluck N, Li D, et al. GCN5 is a required cofactor for a ubiquitin ligase that targets NF- $\kappa$ B/RelA. *Genes Dev* 2009;23:849-861.
- [11] Ryo A, Suizu F, Yoshida Y, et al. Regulation of NF- $\kappa$ B signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA. *Mol Cell* 2003;12:1413-1426.
- [12] Maine GN, Mao X, Komarck CM, et al. COMMD1 promotes the ubiquitination of NF- $\kappa$ B subunits through a Cullin-containing ubiquitin ligase. *EMBO Journal* 2007;26:436-447.
- [13] Geng H, Wittwer T, Dittrich-Breiholz O, et al. Phosphorylation of NF- $\kappa$ B p65 at Ser468 controls its COMMD1-dependent ubiquitination and target gene-specific proteasomal elimination. *EMBO Rep* 2009;10:381-386.
- [14] Burstein E, Hoberg JE, Wilkinson AS, et al. COMMD proteins: A novel family of structural and functional homologs of MURR1. *J Biol Chem* 2005;280:22222-22232.
- [15] Ganesh L, Burstein E, Guha-Niyogi A, et al. The gene product Murr1 restricts HIV-1 replication in resting CD4<sup>+</sup> lymphocytes. *Nature* 2003;426:853-857.
- [16] van de Sluis B, Rothuizen J, Pearson PL, et al. Identification of a new copper metabolism gene by positional cloning in a purebred dog population. *Hum Mol Genet* 2002;11:165-173.
- [17] Biasio W, Chang T, McIntosh CJ, et al. Identification of Murr1 as a regulator of the human  $\alpha$ 1 epithelial sodium channel. *J Biol Chem* 2004;279:5429-5434.
- [18] Drevillon L, Tanguy G, Hinzpeter A, et al. COMMD1-Mediated Ubiquitination Regulates CFTR Trafficking. *PLoS One* 2011;6:e18334.
- [19] Smith L, Litman P, Liedtke CM. COMMD1 interacts with the COOH terminus of NKCC1 in Calu-3 airway epithelial cells to modulate NKCC1 ubiquitination. *Am J Physiol Cell Physiol* 2013;305:C133-C146.
- [20] van de Sluis B, Mao X, Zhai Y, et al. COMMD1 disrupts HIF-1 $\alpha$ /b dimerization and inhibits human tumor cell invasion. *J Clin Invest* 2010;120:2119-2130.
- [21] Fu J, Wolfs MG, Deelen P, et al. Unraveling the regulatory mechanisms underlying tissue-dependent genetic variation of gene expression. *PLoS Genet* 2012;8:e1002431.
- [22] Fehrmann RS, Jansen RC, Veldink JH, et al. Trans-eQTLs reveal that independent genetic variants associated with a complex phenotype converge on intermediate genes, with a major role for the HLA. *PLoS Genet* 2011;7:e1002197.
- [23] van de Sluis B, Muller P, Duran K, et al. Increased activity of hypoxia-inducible factor 1 is associated with early embryonic lethality in *Commd1* null mice. *Mol Cell Biol* 2007;27:4142-4156.

- [24] Vonk WI, Bartuzi P, de Bie P, et al. Liver-specific *Commd1* knockout mice are susceptible to hepatic copper accumulation. *PLoS One* 2011;6:e29183.
- [25] Mao X, Gluck N, Chen B, et al. Copper metabolism MURR1 domain containing 1 (COMMD1) regulates Cullin-RING ligases by preventing Cullin-associated NEDD8-dissociated (CAND1) binding. *J Biol Chem* 2011;286:32355-32365.
- [26] Starokadomskyy P, Gluck N, Li H, et al. CCDC22 deficiency in humans blunts activation of proinflammatory NF- $\kappa$ B signaling. *J Clin Invest* 2013;123:2244-2256.
- [27] Barish GD, Yu RT, Karunasiri M, et al. Bcl-6 and NF- $\kappa$ B cistromes mediate opposing regulation of the innate immune response. *Genes Dev* 2010;24:2760-5.
- [28] Reveille JD, Sims AM, Danoy P, et al. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat Genet* 2010;42:123-7.
- [29] Okada Y, Terao C, Ikari K, Kochi Y, et al. Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. *Nat Genet* 2012;44:511-6.
- [30] Kenny EE, Pe'er I, Karban A, et al. A genome-wide scan of Ashkenazi Jewish Crohn's disease suggests novel susceptibility loci. *PLoS Genet* 2012;8:e1002559.
- [31] Anderson CA, Boucher G, Lees CW, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet* 2011;43:246-252.
- [32] Franke A, McGovern DP, Barrett JC, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet* 2010;42:1118-1125.
- [33] Consortium TEP. A user's guide to the encyclopedia of DNA elements (ENCODE). *PLoS Biol* 2011;9:e1001046.
- [34] Kaikkonen MU, Spann NJ, Heinz S, et al. Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. *Mol Cell* 2013;51:310-25.
- [35] Nicolae DL, Gamazon E, Zhang W, et al. Trait-associated SNPs are more likely to be eQTLs: annotation to enhance discovery from GWAS. *PLoS Genet* 2010;6:e1000888.
- [36] Greten FR, Eckmann L, Greten TF, et al. IKK $\beta$  links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 2004;118:285-296.
- [37] Bollrath J, Pesse TJ, von Burstin VA, et al. gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. *Cancer Cell* 2009;15:91-102.
- [38] Bollrath J, Greten FR. IKK/NF- $\kappa$ B and STAT3 pathways: central signalling hubs in inflammation-mediated tumour promotion and metastasis. *EMBO Rep* 2009;10:1314-1319.
- [39] Weiss KH, Lozoya JC, Tuma S, et al. Copper-Induced Translocation of the Wilson Disease Protein ATP7B Independent of Murr1/ COMMD1 and Rab7. *Am J Pathol* 2008;173:1783-1794.
- [40] Miyayama T, Hiraoka D, Kawaji F, et al. Roles of COMM-domain-containing 1 in stability and recruitment of the copper-transporting ATPase in a mouse hepatoma cell line. *Biochemical Journal* 2010;429:53-61.
- [41] Chang T, Ke Y, Ly K, et al. COMMD1 regulates the delta epithelial sodium channel (dENaC) through trafficking and ubiquitination. *Biochem Biophys Res Commun* 2011;411:506-511.
- [42] Nenci A, Becker C, Wullaert A, et al. Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature* 2007;446:557-561.
- [43] Grivninkov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010;140:883-899.
- [44] Popivanova BK, Kitamura K, Wu Y, et al. Blocking TNF- $\alpha$  in mice reduces colorectal carcinogenesis associated with chronic colitis. *J Clin Invest* 2008;118:560-570.
- [45] Kabakchiev B, Silverberg MS. Expression quantitative trait loci analysis identifies associations between genotype and gene expression in human intestine. *Gastroenterology* 2013;144:1488-96, 1496 e1-3.
- [46] Rivas MA, Beaudoin M, Gardet A, et al. Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease. *Nat Genet* 2011;43:1066-1073.

## SUPPLEMENTARY MATERIALS AND METHODS

**Mouse Strains:** Mice were housed in barrier facilities and fed irradiated standard diet. All animal procedures were approved by the Care and Use Committee. The conditional *Commd1* allele was recently described [1]. LysM-Cre ‘knockin’ mice were obtained from the Jackson laboratory (Bar Harbor, ME). Villin-Cre mice were kindly provided by Dr Eric Fearon.

**Sepsis Models:** Cecal ligation and puncture was performed as previously described [2]. Overall, the surgical time was kept to fewer than 15-20 minutes. After surgery, the mice were housed individually and monitored in a blinded manner (ie, without knowledge of the specific genotypes). This was performed every hour for the first 4-6 hours, then every 2 hours for the next 6 hours, and, finally, every 4-6 hours until the conclusion of the experiment or the need of euthanasia arose (based on morbidity criteria) (Supplementary Table 5). For the LPS model, LPS (*Escherichia coli* 055:B5, Sigma, St. Louis, MO) was diluted in water to 0.2 mg/mL and placed in a water bath sonicator (model FS20, Fisher, Waltham, MA) for 15-30 minutes at room temperature before use. After sonication, the LPS was filtered (0.2  $\mu$ m pore) and injected intraperitoneally (0.1 mg/mouse). After injection, the mice were monitored similarly for morbidity until the conclusion of the experiment (Supplementary Table 5) Bacterial cultures were performed as previously described [3] and tissue RNA was extracted as noted later. Blood also was collected through orbital puncture, and plasma was separated using EDTA (5 mmol/L).

**Acute and Chronic DSS-Induced Colitis:** For the acute colitis model, DSS (Fisher) was administered in the drinking water at a concentration of 3 g/dL (3%). Freshly prepared DSS drinking solution was replaced every 5 days. Mice were housed in individual cages and evaluated daily for their weight and the presence of diarrhea or bleeding to calculate a disease activity index (Supplementary Table 6), which was based on the scoring system reported by Cooper et al [4]. Evaluation for occult bleeding included Hemocult™ testing (Beckman Coulter, Brea, CA). Treatment was given for up to 10 days and animals showing severe distress were euthanized at any point during the experiment.

For the chronic colitis and cancer model, the animals were given azoxymethane intraperitoneally (AOM, 10 mg/kg, Sigma). After 7 days, DSS was given in the drinking water over 4 days at a concentration of 2.5% followed by 10 days of regular water. This was repeated for 3 cycles and at the end of 7 or 10 weeks, the animals were euthanized, and the spleen and colon were harvested. The organ length and weight were determined and the colon was opened along the mesenteric side, and tumors were counted using a dissecting microscope (Zeiss Stemi 2000-C, Jena, Germany). Tumor volumes were calculated using the following formula: (width<sup>2</sup>) x (height /2).

**Histology and Immunohistochemistry:** At the time of euthanasia, the colon was dissected and opened along the mesenteric side. The open colon was transected longitudinally with

one half rolled onto itself as a Swiss roll and fixed in 4% paraformaldehyde (in phosphate-buffered saline). After paraffin embedding and sectioning, the tissue was stained with H&E. The second half was used for RNA extraction as described later. A histologic severity index [4] was used to score colonic sections from mice with acute DSS colitis (Supplementary Table 7). In chronic colitis/dysplasia models (azoxymethane/DSS at 7 weeks), Swiss-roll sections of the colon were assessed for the presence of architectural distortion, dysplasia, multifocal dysplasia, or high-grade dysplasia according to standard clinical pathology criteria. The number of individual foci was not quantitated by this approach. All histopathologic analyses were performed in a blinded fashion by a gastrointestinal pathologist (S.D.M.). For CD68 staining, a tissue microarray was generated using random colonic tissue cores obtained from blocks previously prepared from mice with acute colitis. After constructing the microarrays, 5  $\mu$ m sections were subjected to CD68 staining (clone F8-11; Biolegend, San Diego, CA). Staining was performed on a Ventana Benchmark XT Automated IHC Stainer with Mild CC1 (Cell Conditioning 1) and 32 minutes of primary antibody incubation at a 1:25 dilution (in Chemmate Ab diluent, Dako, Carpinteria, CA). The detection was performed with an Ultraview Universal DAB detection Kit (cat. 760-500; Ventana, Tucson, AZ). The slides were counterstained with Mayer's hematoxylin. Images then were analyzed using the ImageJ software (National Institutes of Health, Bethesda, MD) and cell counts were normalized to the surface area using the surface of a microscopic field as a reference. Co-staining for COMMD1 [5] and F4/80 (11-4801; eBioscience, San Diego, CA) was performed using frozen sections of mouse colon tissue embedded in optimum cutting temperature (OCT) using a previously reported protocol.

**Multiplex Cytokine Measurements:** EDTA-Plasma was separated from blood samples by centrifugation and stored at  $-80^{\circ}\text{C}$ . Full-thickness colonic tissue (1 cm of rectum) was harvested from colitic mice and cultured in 12-well cell culture dish with 1mL of complete Dulbecco's modified Eagle medium culture media. The supernatant was collected 24 hours later, centrifuged and stored at  $-80^{\circ}\text{C}$  until measurement. Cytokine measurements were performed using Milliplex MAP beads (Millipore) and run on Luminex Magpix with the Luminex xPONENT software (Luminex, Austin, TX). Data analysis was performed using Milliplex Analyst.

**Cell Isolation and Cell Culture:** The generation and immortalization of mouse embryo fibroblasts (MEFs) from the *Comm1* floxed mouse has been reported previously [6]. These cells were maintained in high-glucose Dulbecco's modified Eagle medium containing 10% fetal calf serum and treated with MG-132 (20  $\mu$ mol/L for 3 h) for precipitation of ubiquitinated RelA, as described later. BMDMs were generated by isolation of bone marrow cells from long bones, which were cultured in Roswell Park Memorial Institute (RPMI) media supplemented with 10% fetal calf serum, antibiotics (100  $\mu$ g/mL penicillin G and 100  $\mu$ g/mL streptomycin), and Fungizone (Fisher), in the presence of granulocyte-



macrophage colony-stimulating factor (20 ng/mL; Peprotech, Rocky Hill, NJ). Myeloid differentiation was allowed to proceed for 10 days. In specific experiments, cells were treated with LPS (10 ng/mL, *E. coli* 026:B6, Sigma). Hypoxia conditions (3% oxygen) were established in HeraCell incubators. Splenocytes, thymocytes, or mesenteric lymph node (MLN) cells were isolated according to standard protocols. Briefly, after removing the respective organs and placing them on a sterile dish containing RPMI, the tissue was manually ground and strained through a 40  $\mu$ m tissue strainer. After centrifugation (300g for 5 minutes at 4°C), the cell pellet was resuspended in 2 mL of Ammonium-Chloride-Potassium (ACK) buffer (150mM NH<sub>4</sub>Cl, 10mM KHCO<sub>3</sub>, 0.1mM EDTA, pH<sup>+</sup> 7.2) to lyse red blood cells. After 3 minutes at room temperature, the ACK lysis was neutralized by adding 10 mL of fluorescence-activated cell sorter (FACS) media buffer (0.01% Sodium azide, 2.5% fetal calf serum in phosphate-buffered saline). This cell suspension was strained again through the tissue strainer to obtain a single-cell suspension, which was pelleted by centrifugation. Gastrointestinal epithelial cells were isolated according to a previously described method [7]. Briefly, the entire colon, small bowel, or stomach was harvested and flushed clean with 1 mmol/L dithiothreitol (DTT) in Krebs-Ringer Bicarbonate (KRB) buffer (10 mmol/L D-Glucose, 0.5 mmol/L MgCl<sub>2</sub>, 4.6 mmol/L KCl, 120 mmol/L NaCl, 0.7 mmol/L Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mmol/L NaH<sub>2</sub>PO<sub>4</sub>, 15 mmol/L NaHCO<sub>3</sub>). The lumen was filled with KRB buffer supplemented with 10 mmol/L EDTA and 1 mmol/L DTT, and both ends were ligated. The ligated colon was placed in a 50mL conical tube with 30 mL of KRB buffer and placed at 37°C for 20 minutes with agitation. The sealed intestinal segment was palpated gently to loosen the cells, then cut on one end to drain the contents a 1.7mL microfuge tube. The cells were collected by centrifugation at 300g for 5 minutes and rinsed once with KRB buffer.

**Flow Cytometry:** Cells were resuspended in 2 mL of FACS media buffer and the cells from the mesenteric lymph node (MLN) were resuspended in 600  $\mu$ L. For antibody staining, 100  $\mu$ L of each cell suspension was incubated on ice with the following primary antibodies: anti-B220-PE (BD Pharmingen, San Jose, CA), anti-CD3-PE (BD Pharmingen), anti-CD4-perCP (BD Pharmingen), anti-CD8a-APC (BD Pharmingen), anti-Ly6G-APC (eBioscience), anti-Ly6C- PerCP-Cy5.5 (eBioscience), anti-CD11c-APC (BD Pharmingen), anti-CD11b-PE (eBioscience), anti-F4/80-FITC (eBioscience) and corresponding isotype controls. After 30 minutes, the cells were pelleted by centrifugation (300g for 5 minutes at 4°C) and washed twice in 500  $\mu$ L of FACS media buffer. Flow cytometric analysis was performed using FACSCalibur (BD Biosciences, Mountain View, CA) and analyzed using FlowJo software (version 7.6.5, FlowJo, Ashland, OR).

**Protein Extraction, Immunoblotting and Immunoprecipitation:** Cell lysate preparation, nuclear extract preparation, immunoprecipitation, denatured immunoprecipitation and immunoblotting were performed as previously described [5, 8, 9]. Protein lysates from mouse tissues were prepared after snap freezing the samples in liquid nitrogen. After mincing



with a razor blade, tissue pieces were submerged in lysis buffer (50 mmol/L Tris-HCl pH<sup>+</sup> 7.5, 250 mmol/L NaCl, 3 mmol/L EDTA, 3 mmol/L EGTA, 1% Triton X-100, 0.5% NP40, 10% glycerol, 25 mmol/L Na-pyrophosphate) supplemented with protease inhibitor tablets (Roche, San Francisco, CA). Thereafter, tissue homogenization was performed using a 2mL Douncer and a cleared lysate (supernatant after 10,000g centrifugation) was used for further analysis. The following antibodies were used for Western blotting in this study:  $\beta$ -Actin (Sigma, A5441), COMMD1 [5], COMMD9 [10], I $\kappa$ B- $\alpha$  (Upstate Biotechnologies, 06-494), phosphor-I $\kappa$ B- $\alpha$  (Cell signaling, 9246), RelA (Santa Cruz Biotechnology, sc-372 and sc-8008), and ubiquitin (Cell Signaling, 3936).

***RNA Extraction and Quantitative Real-Time Reverse-Transcription Polymerase Chain***

***Reaction:*** In most cases, RNA was extracted using TRIzol (Invitrogen, Grand Island, NY) according to the manufacturer's instructions. In the case of colonic RNA from colitic mice, we obtained better results by first stabilizing the freshly isolated tissue in RNAlater (Qiagen, Hilden, Germany), followed by RNA extraction using the RNeasy chromatography method (Qiagen), according to the manufacturer's instructions. RNA (5  $\mu$ g) was used for complementary DNA synthesis using the Superscript III strand synthesis system (Invitrogen). Real-time polymerase chain reaction was performed in an Eppendorf real-time polymerase chain reaction system. In most cases, SYBR Greenbased detection (Invitrogen) was used using gene specific primers, as noted in Supplementary Table 8. Experiments were performed in triplicate, data were normalized to housekeeping genes, and the relative abundance of transcripts was calculated by the comparative  $\Delta\Delta$ Ct method.

***Microarray Analysis:*** The primary data for this analysis are available in the Gene Expression Omnibus (GEO) database at National Center for Biotechnology Information (NCBI, Accession GSE53368). The Whole Mouse Genome Oligo Microarray V2 (G4846A, ID 026655, Agilent Technologies; Santa Clara, CA) used in this study contains 44,397 oligonucleotide probes covering the entire murine transcriptome. Synthesis of Cy3-labeled complementary RNA was performed with the Quick Amp Labeling kit, one color (5190-0442, Agilent Technologies) according to the manufacturer's recommendations. Complementary RNA fragmentation, hybridization, and washing steps also were performed exactly as recommended per the One-Color Microarray-Based Gene Expression Analysis Protocol V5.7. Slides were scanned on the Agilent Micro Array Scanner G2565CA (pixel resolution, 5  $\mu$ m; bit depth, 20). Data extraction was performed with the Feature Extraction Software V10.7.3.1 by using the recommended default extraction protocol file: GE1\_107\_Sep09.xml. The singlechannel data generated by the Feature Extraction software were normalized and analyzed in Genespring GX software, version 12.0 (Agilent Technologies). Low expression values were increased to the constant value 15 (defined as the threshold) to minimize falsepositive ratios. Data were log<sub>2</sub> transformed and normalized to 75th percentile of each array according to the standard procedure in Genespring GX.

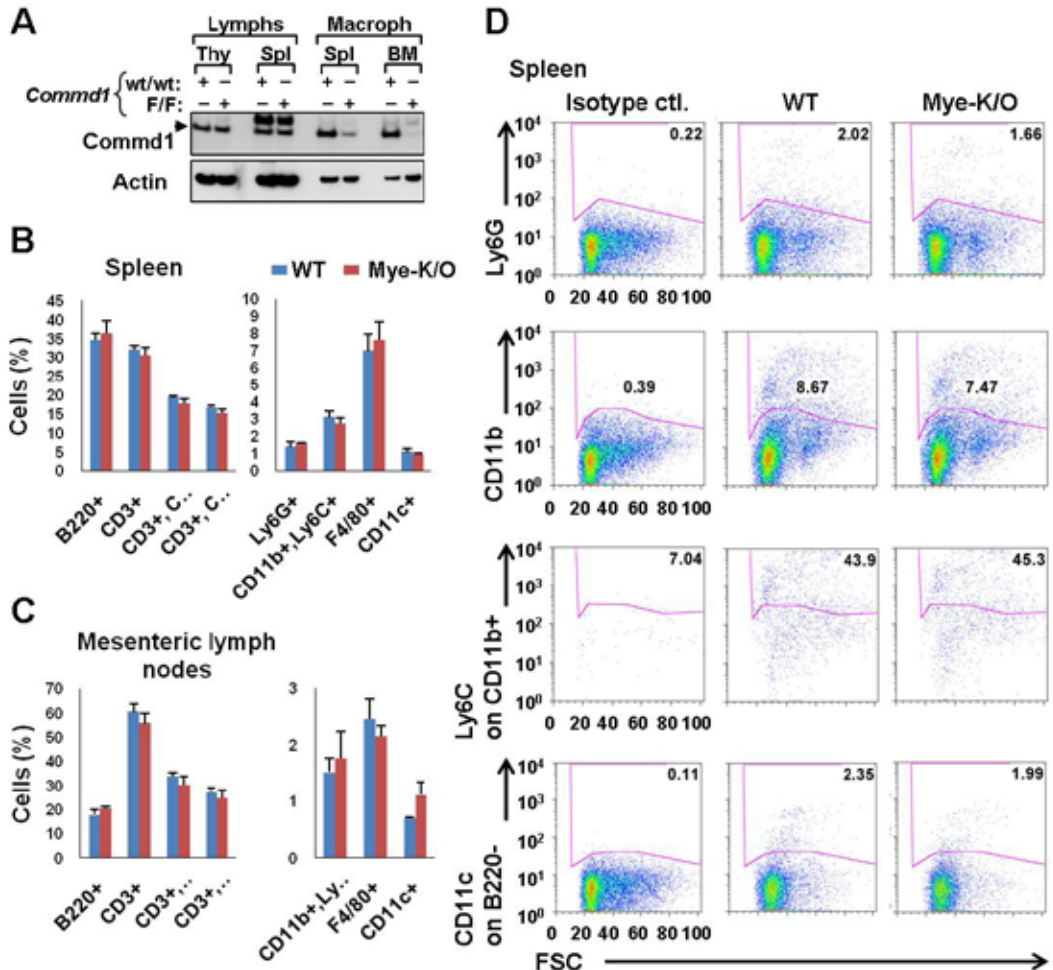
Only probes referring to transcripts of annotated genes were considered and Agilent probe annotation was used. In cases of multiple probes per gene the value from that probe with the highest arithmetic mean intensity across all 12 hybridizations was selected. Genes were considered as measurable when the arithmetic mean of (antilog<sub>2</sub>-retransformed) intensity values calculated from all 12 samples was greater than 100 and mean intensity in at least 1 of 6 averaged conditions was greater than 50. Entities showing technical impairments were excluded from analyses. By using these criteria, of 44,397 probes, 18,682 probes corresponding to 11,441 genes were selected for further analysis. Genes were considered as regulated by LPS or by Commd1 KO according to the following criteria: the foldchange of mean values in at least 1 of 3 ratios was greater than 3-fold and regulation was consistent with the same trend in each single (nonaveraged) comparison. The following ratios were calculated: Commd1 KO vs WT, LPS 3 hours WT vs WT, LPS 24 hours WT vs WT. In the resulting gene set Commd1-dependent genes were defined by at least 2-fold differences of the following ratios of mean intensity values: deregulated basal expression (ratio Commd1 KO vs WT), deregulated less than 3 hours LPS treatment (Commd1 KO LPS 3 h vs WT LPS 3 h), deregulated less than 24 hours LPS treatment (Commd1 KO LPS 24 h vs WT LPS 24 h). The ratio values of the resulting COMMD1-dependent 225 genes were analyzed by hierarchical cluster analysis using MeV MultiExperimentViewer, version 4.8.1, 2011 (available: [www.tm4.org](http://www.tm4.org)) with cluster settings “average” as linkage method and “Manhattan distance” as distance measure.

Gene function analysis was performed using the DAVID (v6.7) software platform (maintained by National Institute of Allergy and Infectious Disease (NIAID) and available at <http://david.abcc.ncifcrf.gov/home.jsp>). Functional annotation was performed using the KEGG database (KEGG Mapper v1.6, released October 1, 2013). The regulation of genes of interest by NF-κB was ascertained by comparing each gene to 2 partially redundant data sets: a curated database of published NF-κB gene targets (publicly available and maintained at [www.NF-kB.org](http://www.NF-kB.org)), and, second, a chromatin immunoprecipitation (ChIP) seq data set of RelA-regulated genes in LPS-stimulated BMDMs [11].

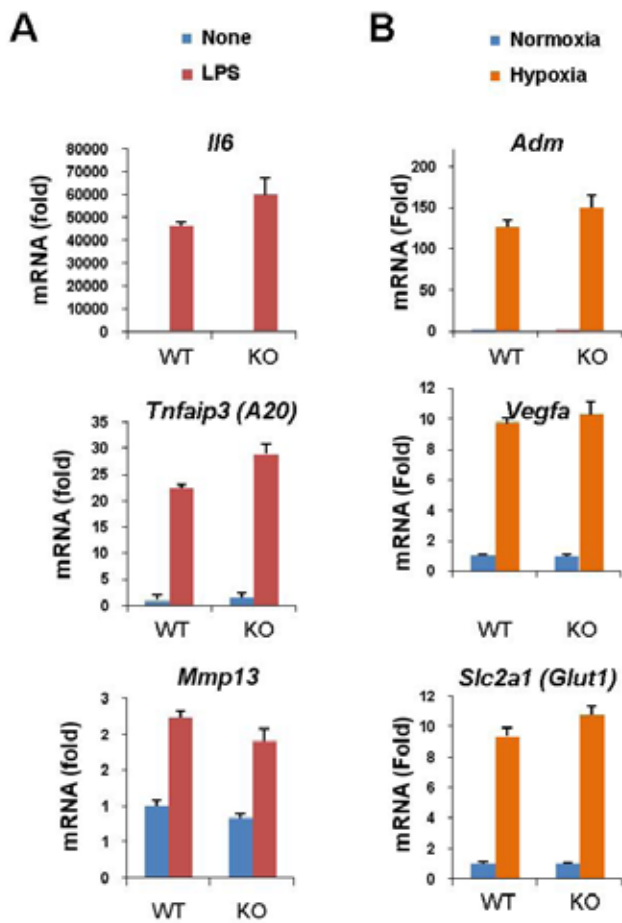
## REFERENCES

- [1] Vonk WI, Bartuzi P, de Bie P, et al. Liver-specific *Comm1d1* knockout mice are susceptible to hepatic copper accumulation. *PLoS One* 2011;6:e29183.
- [2] Toscano MG, Ganea D, Gamero AM. Cecal ligation puncture procedure. *J Vis Exp* 2011;pii: 2860.
- [3] Kirkland D, Benson A, Mirpuri J, et al. B cell-intrinsic MyD88 signaling prevents the lethal dissemination of commensal bacteria during colonic damage. *Immunity* 2012;36:228-38.
- [4] Cooper HS, Murthy SN, Shah RS, et al. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 1993;69:238-249.
- [5] Burstein E, Hoberg JE, Wilkinson AS, et al. COMMD proteins: A novel family of structural and functional homologs of MURR1. *J Biol Chem* 2005;280:22222-22232.
- [6] Vonk WI, de Bie P, Wichers CG, et al. The copper-transporting capacity of ATP7A mutants associated with Menkes disease is ameliorated by COMMD1 as a result of improved protein expression. *Cell Mol Life Sci* 2012;69:149-63.
- [7] Ahnen DJ, Reed TA, Bozdech JM. Isolation and characterization of populations of mature and immature rat colonocytes. *Am J Physiol* 1988;254:G610-G621.
- [8] Burstein E, Ganesh L, Dick RD, et al. A novel role for XIAP in copper homeostasis through regulation of MURR1. *EMBO Journal* 2004;23:244-254.
- [9] Mao X, Gluck N, Li D, et al. GCN5 is a required cofactor for a ubiquitin ligase that targets NF- $\kappa$ B/RelA. *Genes Dev* 2009;23:849-861.
- [10] Starokadomskyy P, Gluck N, Li H, et al. CCDC22 deficiency in humans blunts activation of proinflammatory NF- $\kappa$ B signaling. *J Clin Invest* 2013;123:2244-2256.
- [11] Barish GD, Yu RT, Karunasiri M, et al. Bcl-6 and NF- $\kappa$ B cistromes mediate opposing regulation of the innate immune response. *Genes Dev* 2010;24:2760-5.
- [12] Ramirez-Carrozzi VR, Nazarian AA, Li CC, et al. Selective and antagonistic functions of SWI/SNF and Mi-2b nucleosome remodeling complexes during an inflammatory response. *Genes Dev* 2006;20:282-296.
- [13] Zhao A, Urban JF, Jr., Anthony RM, et al. Th2 cytokine-induced alterations in intestinal smooth muscle function depend on alternatively activated macrophages. *Gastroenterology* 2008;135:217-225 e1.
- [14] Moreno R, Sobotzik JM, Schultz C, et al. Specification of the NF- $\kappa$ B transcriptional response by p65 phosphorylation and TNF-induced nuclear translocation of IKK $\epsilon$ . *Nucleic Acids Res* 2010;38:6029-6044.
- [15] Mastrogiannaki M, Matak P, Keith B, et al. HIF-2 $\alpha$ , but not HIF-1 $\alpha$ , promotes iron absorption in mice. *J Clin Invest* 2009;119:1159-1166.

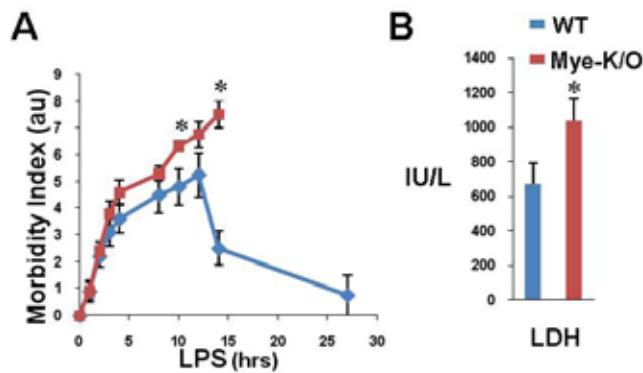
## SUPPLEMENTARY FIGURES AND TABLES



**Figure S1 - Baseline characterization of Mye-K/O mice.** (A) *Commd1* deficiency in myeloid cells of Mye-K/O mice was verified by western blot in myeloid cells derived from bone marrow cells or adherent splenocytes after 10 day culture. Lymphocyte populations (primary thymocytes and non-adherent splenocytes) are shown as controls. (B) and (C) Spleen and MLN populations were not affected in Mye-K/O mice. Various cell populations were determined using flow cytometry for the indicated surface markers ( $n=3$  for each group). (D) Flow cytometry immunophenotypic analysis of spleenocytes was performed using markers for granulocytes (Ly6G<sup>+</sup>), macrophages (CD11b<sup>+</sup> and Ly6C<sup>+</sup>), activated phagocytes (F4/80<sup>+</sup>), dendritic cells (CD11c<sup>+</sup>) and B lymphocytes (B220<sup>+</sup>). Representative plots of cells expressing the indicated markers are shown.



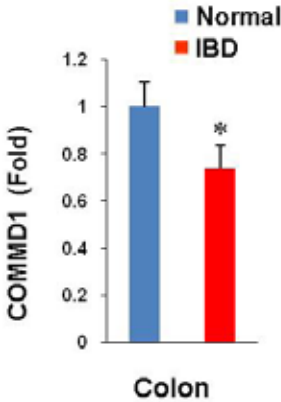
**Figure S2 - Commd1 deficiency did not affect expression of certain NF- $\kappa$ B and hypoxia gene targets.** (A) and (B) Bone-marrow derived myeloid cells (2 mice per group, with triplicate cultures each) were stimulated with LPS or exposed to hypoxia conditions and expression of inducible genes were determined by qRT-PCR.



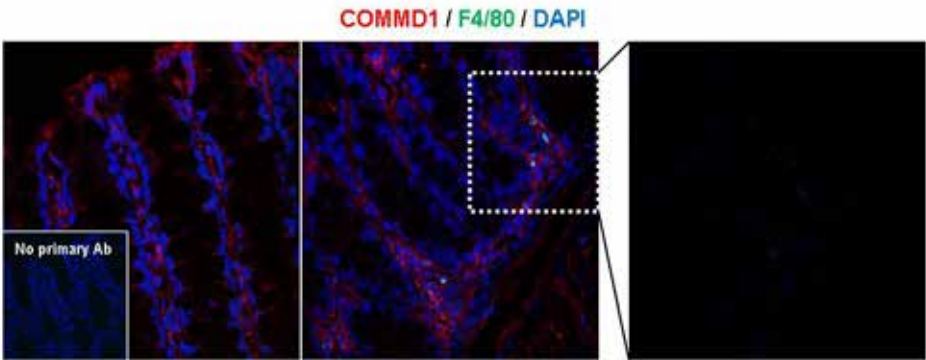
**Figure S3 - Mye-K/O mice have a more severe inflammatory response to LPS.** (A) After intraperitoneal LPS administration, a morbidity index (Table S5) was calculated at the indicated time points. Mye-K/O mice demonstrated greater morbidity compared to wild-type (WT) littermate controls. (B) Plasma levels of LDH were elevated in Mye-K/O mice (n=8) compared to WT controls (n=6). \* p < 0.05

Organ	Genes	Average (fold)		p value
		WT	Mye-K/O	
Kidney	Tnf	5.9	26.6	0.002
	Ccl5	23.8	109.2	0.002
	Il1b	5.8	12.1	0.011
	Il6	475.3	1830.1	0.004
	Saa3	169.6	429.9	0.044
Liver	Tnf	6.3	18.1	0.030
	Ccl5	5.7	23.8	0.006
	Il1b	7.6	26.8	0.028
	Il6	26.5	120.8	0.053
	Saa3	318.2	355.8	0.307
Lung	Tnf	3.5	6.6	0.020
	Ccl5	3.1	9.9	0.004
	Il1b	12.6	21.1	0.063
	Il6	72.8	191.9	0.086
	Saa3	274.0	932.1	0.038

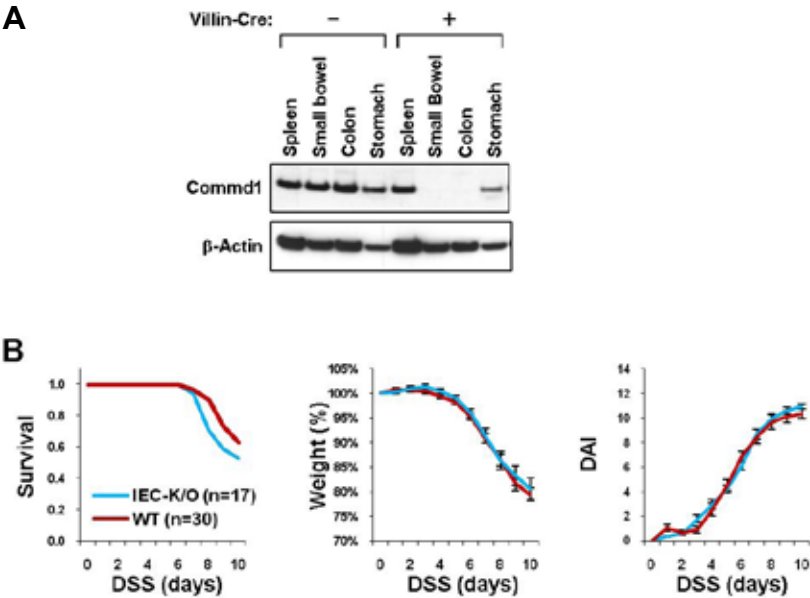
**Figure S4 - Pro-inflammatory gene expression after LPS challenge was more pronounced in Mye-K/O mice.** After RNA isolation, gene expression was evaluated in different organs by qRT-PCR. Highlighted in green are genes that displayed a statistically significant difference (p value < 0.05).



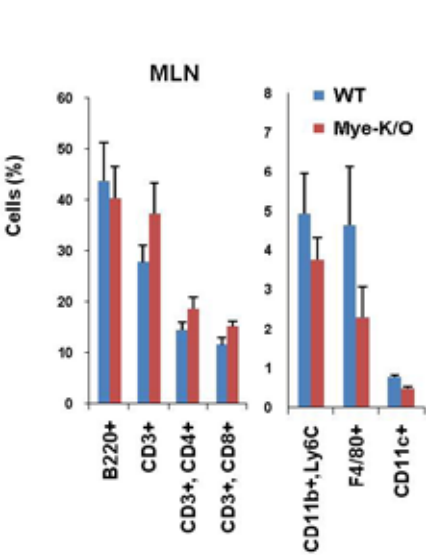
**Figure S5 - COMMD gene suppression in IBD patients.** COMMD1 expression in the colonic mucosa was reduced in a cohort of IBD patients in the USA (22 normal controls and 17 patients with IBD, 9 of which had CD and 8 with UC). \* p =0.04.



**Figure S6 – Commd1 is expressed in epithelium and lamina propria macrophages of the colon.** Frozen sections of mouse colon tissue were stained with Commd1-specific antibody and counterstained with the macrophage marker F4/80. DAPI staining was used to delineate the nuclei. A negative control not including the primary antibodies is also shown. Magnification bar equals 100 µm.



**Figure S7 - Commd1 deficiency in the intestinal epithelium did not promote affect acute colitis severity.** (A) Deletion of Commd1 in intestinal epithelial cells of Commd1F/F, Vilin-Cre mice (IEC-K/O) was demonstrated by western blot analysis using cell lysates from small bowel epithelial cells and colonocytes; splenocytes, and gastric epithelial cells serve as controls. (B) No difference was found between IEC-K/O and WT mice in terms of survival rate, weight loss or disease activity in an acute model of colitis.



**Figure S8 - Lymphocyte and macrophage populations during acute colitis.** Lymphocyte and myeloid populations were not substantially different in the MLN of Mye-K/O mice compared to controls (n=3 in each group). Flow cytometry was utilized to identify the indicated cell populations as before.



Gene designation	Cytokine name	Average (pg/mL)		p value
		WT	Mye-K/O	
<i>Csf3</i>	G-CSF	7472.7	7986.6	0.3586
<i>Csf2</i>	GM-CSF	188.5	172.7	0.3672
<i>Ifng</i>	IFN- $\gamma$	63.3	109.5	0.0789
<i>Tnf</i>	TNF	21.2	38.7	0.0124
<i>Il1a</i>	IL-1 $\alpha$	82.3	222.3	0.0653
<i>Il1b</i>	IL-1 $\beta$	54.7	129.6	0.0445
<i>Il2</i>	IL-2	1.9	2.8	0.0344
<i>Il4</i>	IL-4	1.5	1.4	0.3763
<i>Il5</i>	IL-5	72.1	51.5	0.2647
<i>Il6</i>	IL-6	3203.9	3365.6	0.4423
<i>Il7</i>	IL-7	4.1	3.1	0.2399
<i>Il9</i>	IL-9	92.5	96.1	0.3726
<i>Il10</i>	IL-10	71.6	96.1	0.0813
<i>Il12b</i>	IL-12 (p40)	5.2	6.1	0.1725
* <i>Il12a/b</i> dimer	IL-12 (p70)	8.8	10.8	0.1337
<i>Il13</i>	IL-13	114.9	140.6	0.1807
<i>Il15</i>	IL-15	46.4	39.7	0.2757
<i>Il17a</i>	IL-17	42.0	63.3	0.1462
<i>Ccl2</i>	MCP-1	2375.1	2924.0	0.2864
<i>Ccl3</i>	MIP-1a	39.1	91.3	0.0212
<i>Ccl4</i>	MIP-1b	33.6	47.4	0.0845
<i>Ccl5</i>	RANTES	17.6	22.8	0.1004
<i>Cxcl1</i>	KC	4297.9	4186.6	0.4518
<i>Cxcl2</i>	MIP-2	9866.2	13223.8	0.0372
<i>Cxcl10</i>	IP-10	199.7	279.6	0.1052

**Figure S9 - Commd1 deficiency leads to greater cytokines secretion from inflamed colonic tissue.**

Full thickness colonic tissue from mice with chronic DSS colitis were harvested and cultured overnight (WT, n=15; Mye-K/O, n=14).

The released of cytokines from these tissues into the culture media was evaluated by a multiplex bead-based assay. Marked in green are statistically significant differences between the groups (p value < 0.05).

**Table S5: Morbidity score for sepsis models**

<u>APPEARANCE</u>	
0	Normal
1	Lack of grooming
2	Coat rough, possible nasal or ocular discharge
3	Coat very rough, abnormal posture, eyes sunken and glazed
<u>CLINICAL SIGNS</u>	
0	Normal
1	Diarrhea, constipation
2	Respiratory rate altered, respiratory depth altered, skin tents
3	Cyanotic extremities, labored breathing
<u>UNPROVOKED BEHAVIOR</u>	
0	Normal
1	Minor changes
2	Abnormal behavior, less mobile, less alert, inactive when activity expected
3	Paralysis, inability to remain upright, shivering, convulsion
<u>Total score:</u> Sum of (Domain scores) for each domain in the scoring system	

**Table S6: Disease activity index in the acute DSS colitis model**

<u>WEIGHT</u> 0 Unchanged 1 Loss of 1-5% 2 Loss of 6-10% 3 Loss of 11-20% 4 Greater than 20% weight loss
<u>STOOL CONSISTENCY</u> 0 Normal 2 Loose stools (not watery) 4 Diarrhea (liquid stool)
<u>BLEEDING</u> 0 None 2 Hemocult positive stools 3 Visible blood on stool 4 Gross bleeding per rectum
<u>Total score:</u> Sum of (Domain scores) for each domain in the scoring system

**Table S7: Histologic score for the acute DSS colitis model**

<u>DOMAIN</u>	<u>INVOLVEMENT</u>
<u>A) CRYPT LOSS</u> 0 Intact 1 Loss of basal one-third of crypt 2 Loss of basal two-thirds of crypt 3 Loss of entire crypt 4 Erosion (loss of entire crypt and surface epithelium)	  

Table S8: Primer sequences used in quantitative PCR analysis

Mouse genes			
Gene target	Sense primer	Antisense primer	Reference
<i>Actb</i>	GGCTGTATTCCGCTCCATCG	CCAGTTGGTAACAATGCCATGT	This study
<i>Adm</i>	TCCGAAAGAAAGTGGAAATAAGTGG	GTCCCGTAGGGTAGCTGCT	PrimerBank ID 6752987b2 <sup>12</sup>
<i>Cd5</i>	GTGCCAGAGTCAAGGAGTAT	CCCACTTCTCTCTGGGTG	PrimerBank ID 6753310a1
<i>Cnd2</i>	GAGTGGAACTGGTAGTGTG	CGCACAGAGCGGATGAAGT	PrimerBank ID 161484598c1
<i>Cd86</i>	TCAATGGGACTGCATATCTGCC	GCCAAAATACTACCAAGCTCACT	This study
<i>Commd1</i>	TGGCGAAGATGAGAGGACTT	GTGATGCCACCTTGCTTTT	
<i>Commd2</i>	Taqman primer and probe set from Applied Biosystems		
<i>Commd3</i>	Taqman primer and probe set from Applied Biosystems		
<i>Commd4</i>	Taqman primer and probe set from Applied Biosystems		
<i>Commd5</i>	Taqman primer and probe set from Applied Biosystems		
<i>Commd6</i>	Taqman primer and probe set from Applied Biosystems		
<i>Commd7</i>	Taqman primer and probe set from Applied Biosystems		
<i>Commd8</i>	Taqman primer and probe set from Applied Biosystems		
<i>Commd9</i>	CC'TCC'TCTGACAAACATCAGC		
<i>Commd10</i>	TGAGAAGTTCCGCCAGAG		
<i>Cxcl1</i>	TGTCAGTGCCTGCAGACCAT	GGAGGGTTTCTCTCCACAC	This study
<i>Emr1</i>	AAAGACTGGATTCTGGGAAGTTTGG	TTGCTCACTCCCAGTTGC	This study <sup>12</sup>
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTG	TTGGCTATGACTTCGGTTTGG	<sup>13</sup>
<i>Il1b</i>	GCTGAAAGCTCTCCACCTCA	CGAGAGTGTGTGGCAGGTTG	This study <sup>12</sup>
<i>Il6</i>	TGGATGCTACCAACTGGAT	TGTAGACCATGTAGTTAGGICA	<sup>14</sup>
<i>Il33</i>	TCCAACTCCAAGATTCCCCG	AGCCACAGGTATTTGTGG	PrimerBank ID 19527000a1
<i>Mx1</i>	AACTGTATCCGACTTCACTCC	GGACTCGGCTTTGTCTTTC	<sup>12</sup>
<i>Saa3</i>	CTGTTCAGAAATTCAAGGGAC	CATGCGTCTCAAGGTTTCCCTGT	<sup>14</sup>
<i>Slc2a1</i>	GGCATGTGCTTCCAGTATGT	ACGAGGAGCACCGTGAAGAT	<sup>15</sup>
<i>Sell</i>	TACATTGCCCAAAGCCCTTAT	CATCGTTCCATTTCGCAGAGTC	PrimerBank ID 6755454a1
<i>Tnf</i>	CCCAAAAGGGATGAGAAGTT	TGGGCTACAGGCTTGTCACCT	<sup>12</sup>
<i>Vegfa</i>	CTTGTTCAAGCGGAGAAAGC	ACATCTGCAAGTACGTTCTGT	PrimerBank ID 6678563a2
Human genes			
Gene target	Sense primer	Antisense primer	Reference
<i>ACTB</i>	GCGGGAAATCGTCCGTGACATT	GATGGAGTTGAAGGTAGTTTCGTG	This study <sup>5</sup>
<i>COMMD1</i>	GCAAATATGGACAGGAATC	CAGAATTTGGTTGACTTTTGAC	
<i>GAPDH</i>	CCCACCTCTCCACCTTTTGAC	TGTTGCTGTAGCCAAATTCGT	This study

